WE CLAIM:

1. A method for detecting a specific nucleotide variation at a defined site in a target nucleic acid polymer wherein a first nucleotide residue is replaced by a second nucleotide residue, comprising the steps of:

(a) hybridizing a detectable amount of a target nucleic acid polymer in single-stranded form with an oligonucleotide primer, the detection step primer, comprising a plurality of nucleotide residues, said primer being complementary to the nucleotide sequence of interest in a region disposed toward the 3' end from the defined site such that when the primer is hybridized to the polymer there are no nucleotide residues between the defined site and the 3' end of the primer that are identical to the first or second nucleotide residues to be detected;

(b) extending the primer using a polymerizing agent in a mixture comprising one or more nucleoside triphosphates wherein the mixture includes at least one nucleoside triphosphate complementary to either the first or second nucleotide residue which comprises means for detecting the incorporation of the nucleoside triphosphate in a nucleic acid polymer, and optionally one or more chain terminating nucleoside triphosphates; and

(c) detecting the incorporation of the nucleoside triphosphate, whereby the identity of the nucleotide residue at the defined site is determined.

AMD. per "B" thru "D" + AMD. per "E"

2. A method for detecting a plurality of specific nucleotide variations at defined sites in a target nucleic acid polymer wherein at least a first nucleotide residue is

replaced by a second nucleotide residue at a first defined site and a third nucleotide residue is replaced by a fourth nucleotide residue at a second defined site, comprising

(a) hybridizing a detectable amount of a target nucleic acid polymer in single-stranded form with a first oligonucleotide primer, the first detection step primer, comprising a plurality of nucleotide residues, said primer being complementary to the nucleotide sequence of interest in a region disposed toward the 3' end from the first defined site such that when the primer is hybridized to the polymer there are no nucleotide residues between the first defined site and the 3' end of the primer that are identical to the first and second nucleotide residues;

(b) extending the first detection step primer using a polymerizing agent in a mixture comprising one or more nucleoside triphosphates wherein the mixture includes at least one nucleoside triphosphate complementary to the first or second nucleotide residue which comprises means for detecting the incorporation of the nucleoside triphosphate in a nucleic acid polymer, and optionally one or more chain terminating nucleoside triphosphates;

(c) detecting the incorporation of the nucleoside triphosphate, whereby the identity of the nucleotide residue at the first defined site is determined;

(d) removing the extended first detection step primer formed in step (c) from the target nucleic acid polymer; and

(e) adding a second detection step primer, said primer being complementary to the nucleotide sequence of interest in a region disposed toward the 3' end from the

second defined site such that when the primer is hybridized to the immobilized polymer there are no nucleotide residues between the second defined site and the 3' end of the primer that are identical to the third or forth nucleotide residues to be detected.

3. A method of detecting in a patient a predisposition to a genetic disorder resulting from a specific nucleotide variation at a defined site in genetic material of the patient wherein a first nucleotide residue is replaced by a second nucleotide residue, comprising the steps of:

(a) obtaining a sample containing a detectable amount of genetic material derived from the patient;

(b) hybridizing the detectable amount of genetic material in a single-stranded form with a first oligonucleotide primer, the first detection step primer, comprising a plurality of nucleotide residues, said primer being complementary to the nucleotide sequence of interest in a region disposed toward the 3' end from the first defined site such that when the primer is hybridized to the genetic material there are no nucleotide residues between the defined site and the 3' end of the primer that are identical to the first and second nucleotide residues;

(c) extending the primer using a polymerizing agent in a mixture comprising one or more nucleoside triphosphates complementary to either the first or second nucleotide residue which comprises means for detecting the incorporation of the nucleoside triphosphate in a nucleic

acid polymer, and optionally one or more chain terminating nucleoside triphosphates; and

(d) detecting the incorporation of the nucleoside triphosphate, whereby the identity of the nucleotide residue at the defined site, and thus whether the patient has a predisposition for the associated genetic disorder is determined.

4. A method according to claim 1, 2 or 3 further comprising the step of immobilizing the target nucleic acid polymer to a solid support prior to step (a).

5. A method according to claims 1, 2 or 3, wherein the primer is complementary to a region of the nucleotide sequence of interest extending toward the 3' end of the target nucleic acid polymer from the nucleotide residue immediately adjacent to the defined site.

6. A method according to claim 1, 2 or 3, wherein the nucleoside triphosphate comprising means for detecting the incorporation of the nucleoside triphosphate in a nucleic acid polymer is a deoxynucleoside triphosphate.

7. A method according to claim 1, 2 or 3, wherein the nucleoside triphosphate comprising means for detecting the incorporation of the nucleoside triphosphate in a nucleic acid polymer is a dideoxynucleoside triphosphate.

8. A method according to claims 1, 2 or 3, wherein the mixture includes a second nucleoside triphosphate

comprising a second means, different from said first means, for detecting the incorporation of the second nucleoside triphosphate in a nucleic acid polymer.

9. A method according to claim 1, 2 or 3, wherein the extended product of step (d) is eluted before determining the incorporation of the incorporated nucleoside triphosphate.

10. A method according to claim 2 or 3, wherein the nucleotide variations are detected in one single step by adding a plurality of detection step primers and differently labelled nucleoside triphosphates identifying the variable nucleotide residues. AMD. pur "E"

11. A method according to claim 1, 2, or 3 wherein the detectable amount of target nucleic acid polymer is obtained by performing a modified amplification reaction wherein at least one amplification primer comprises a first attachment moiety bonded to the primer. → CANC. pur "C"

12. A kit for use in determining specific nucleotide variations in a target nucleic acid polymer comprising in packaged combination

(a) at least one amplification primer comprising an oligonucleotide which is complementary to and hybridizes with a portion of the target nucleic acid polymer and which is effective as a primer for enzymatic nucleic acid polymerization and a first attachment moiety;

(b) at least one detection step primer comprising an oligonucleotide which is complementary to and hybridizes with a portion 3' to a variable nucleotide of the target nucleic acid polymer; and optionally;

(c) at least one solid support comprising a solid matrix and at least one attachment site which is capable of immobilizing the oligonucleotide of the amplification probe through the first attachment moiety; and

(d) at least one nucleoside triphosphate containing means for detecting the incorporation of the nucleoside triphosphate in a nucleic acid polymer.

13. A kit according to claim 12 for use in the identification of the nucleotide variation of apolipoprotein E polymorphism, wherein the detection step primer comprises the sequence 5'-GCG CGG ACA TGG AGG ACG TG.

14. A kit according to claim 12 for use in the identification of the nucleotide variation of apolipoprotein E polymorphism, wherein the detection step primer comprises the sequence 5'-ATG CCG ATG ACC TGC AGA AG.

15. A kit according to claim 12 for use in the identification of the nucleotide variation of apolipoprotein E polymorphism, wherein the detection step primer comprises the sequence 5'- GTA CTG CAC CAG GCG GCC GC.

16. A kit according to claim 12 for use in the identification of the nucleotide variation of apolipoprotein

E polymorphism, wherein the detection step primer comprises the sequence 5'- GGC CTG GTA CAC TGC CAG GC.

17. A kit according to claim 12 for use in the detection of the nucleotide variation in codon 6 of the human 8-globin gene causing sickle cell anemia, wherein the detection step primer comprises the sequence 5'-CAT GGT GCA CCT GAC TCC TG.

18. A kit according to claim 12 for use in the detection of the nucleotide variation in codon 6 of the human 8-globin gene causing sickle cell anemia, wherein the detection step primer comprises the sequence 5'- CAG TAA CGG CAG GCG GCC GC.

19. A kit according to claim 12 for use in the detection of a nucleotide variation in codon 12 of the K-ras gene, wherein the detection step primer comprises the sequence 5'- AAG GCA CTC TTG CCT ACG CCA.

20. A kit according to claim 12 for use in the detection of a nucleotide variation in codon 12 of the K-ras gene, wherein the detection step primer comprises the sequence 5'-AGG CAC TCT TGC CTA CGC CAC.

21. A kit according to claim 12 for use in the detection of a nucleotide variation in codon 12 of the K-ras gene, wherein the detection step primer comprises the sequence 5'- AAC TTG TGG TAG TTG GAG CT.

22. A kit according to claim 12 for use in the detection of a nucleotide variation in codon 12 of the K-ras gene, wherein the detection step primer comprises the sequence 5'- ACT TGT GGI AGT TGG AGC TG.

23. A reagent for detecting the presence of a point mutation in which a normal nucleic acid residue is replaced by an abnormal nucleic acid residue at a defined site within a gene of interest, comprising an oligonucleotide of sufficient length to act as a primer for an enzyme catalyzed chain extension nucleic acid polymerization reaction, said oligonucleotide primer having a sequence which is complementary to a region of the gene of interest beginning with the nucleotide residue immediately adjacent to and toward the 3' end of the gene from the defined site and extending away from the defined site toward the 3' end of the gene whereby enzyme catalyzed chain extension nucleic acid polymerization will commence by adding a nucleic acid residue complementary to either the normal nucleic residue or the abnormal nucleic acid residue.

24. A reagent according to claim 23, wherein the polynucleotide has a length of from 10 - 40 nucleotide residues.

25. A reagent according to claim 24 having the sequence 5'-GCG CGG ACA TGG AGG ACG TG.

26. A reagent according to claim 24 having the sequence 5'-ATG CCG ATG ACC TGC AGA AG.

27. A reagent according to claim 24 having the sequence 5'- GTA CTG CAC CAG GCG GCC GC.

28. A reagent according to claim 24 having the sequence 5'- GGC CTG GTA CAC TGC GAG GC.

29. A reagent according to claim 24 having the sequence 5'-CAT GGT GCA CCT GAC TCC TG.

30. A reagent according to claim 24 having the sequence 5'- CAG TAA CGG CAG GCC GCC GC.

31. A reagent according to claim 24 having the sequence 5'- AAG GCA CTC TTG CCT ACG CCA.

32. A reagent according to claim 24 having the sequence 5'-AGG CAC TCT TGC CTA CGC CAC.

33. A reagent according to claim 24 having the sequence 5'- AAC TTG TGG TAG TTG GAG CT.

34. A method for detecting, at a defined site in the genome of a microorganism, the existence of point mutations leading to altered pathogenicity or resistance to therapy in the microorganisms wherein a first nucleotide residue is replaced by a second nucleotide residue, comprising the steps of:

(a) obtaining a sample containing a detectable amount of genetic material derived from the microorganism;

(b) hybridizing the detectable amount of genetic material in a single-stranded form with an oligonucleotide primer, the detection step primer, comprising a plurality of nucleotide residues, said primer being complementary to the nucleotide sequence of interest in a region disposed toward the 3' end from the defined site such that when the primer is hybridized to the genetic material there are no nucleotide residues between the defined site and the 3' end of the primer that are identical to the first or second nucleotide residues to be detected;

(c) extending the primer using a polymerizing agent in a mixture comprising one or more nucleoside triphosphates wherein the mixture includes at least one nucleoside triphosphate complementary to either the first or second nucleotide residue which comprises means for detecting the incorporation of the nucleoside triphosphate in a nucleic acid polymer, and optionally one or more chain terminating nucleoside triphosphates; and

(d) detecting the incorporation of the nucleoside triphosphate, whereby the identity of the nucleotide residue at the defined site and thus whether a point mutation has occurred is determined.

AMD. per "D" & "E"

35. A method according to claim 34, wherein the microorganism is human immunodeficiency virus.

36. A method according to claim 35, wherein the point mutation is at a site selected from among Asp 67, Lys 70 and Thr 215.

AMD. per "E"

37. A method for detecting cells having a point mutation at a defined site in the genetic material, wherein a first nucleotide residue is replaced by a second nucleotide residue, when said mutated cells are mixed in a cell population with unmutated cells comprising the steps of:

(a) obtaining a detectable quantity of genetic material from the cell population while maintaining the ratio of mutated to unmutated cells;

(b) hybridizing the detectable amount of genetic material in a single-stranded form with an oligonucleotide primer, the detection step primer, comprising a plurality of nucleotide residues, said primer being complementary to the nucleotide sequence of interest in a region ~~disposed toward~~ the 3' end ~~from~~ the defined site such that when the primer is hybridized to the genetic material there are no nucleotide residues between the defined site and the 3' end of the primer that are identical to the first or second nucleotide residues to be detected;

(c) extending the primer using a polymerizing agent in a mixture comprising one or more nucleoside triphosphates wherein the mixture includes at least one nucleoside triphosphate complementary to either the first or second nucleotide residue which comprises means for detecting the incorporation of the nucleoside triphosphate in a nucleic acid polymer, and optionally one or more chain terminating nucleoside triphosphates; and

(d) detecting the incorporation of the nucleoside triphosphate, whereby the identity of the nucleotide residue at the defined site and thus whether mutated cells are present is determined.

AMD. per "C" + "E"

38. A method according to claim 37 wherein the cells are lymphocytes.

39. A method according to the claim 38, wherein the mutated cells are leukemic cells.

~~40. Added per "C"~~

~~41.- 46. Added per "D"~~

~~47. Added per "E"~~

Add 41

add D3